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**Roles of integrins in human induced pluripotent stem cell growth on Matrigel and vitronectin.**

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**Public Summary:**

Human induced pluripotent stem cells (iPSCs) hold promise as a source of adult-derived, patient-specific pluripotent cells for use in cell-based regenerative therapies. However, current methods of cell culture are tedious and expensive, and the mechanisms underlying cell proliferation are not understood. We aim to develop better methods for growing pluripotent stem cells. In this study, we investigated expression and function of iPSC integrin extracellular matrix receptors to better understand the molecular mechanisms of cell adhesion, survival, and proliferation, which are important for growth in culture. We show that iPSC lines generated using the Thomson factors express a repertoire of integrins similar to that of hESCs, with prominent expression of subunits alpha5, alpha6, alphav, beta1, and beta5. Integrin function was investigated in iPSCs cultured without feeder layers on Matrigel or vitronectin, in comparison to human embryonic stem cells. beta1 integrins were required for adhesion and proliferation on Matrigel, as shown by immunological blockade experiments. On vitronectin, the integrin alphavbeta5 was required for initial attachment, but inhibition of both alphavbeta5 and beta1 was required to significantly decrease iPSC proliferation. Furthermore, iPSCs cultured on vitronectin for 9 passages retained normal karyotype, pluripotency marker expression, and capacity to differentiate in vitro. These studies suggest that vitronectin, or derivatives thereof, might substitute for Matrigel in a more defined system for iPSC culture. This represents a simple method that could replace a reagent that is difficult to work with.

**Scientific Abstract:**

Human induced pluripotent stem cells (iPSCs) hold promise as a source of adult-derived, patient-specific pluripotent cells for use in cell-based regenerative therapies. However, current methods of cell culture are tedious and expensive, and the mechanisms underlying cell proliferation are not understood. In this study, we investigated expression and function of iPSC integrin extracellular matrix receptors to better understand the molecular mechanisms of cell adhesion, survival, and proliferation. We show that iPSC lines generated using Oct-3/4, Sox-2, Nanog, and Lin-28 express a repertoire of integrins similar to that of hESCs, with prominent expression of subunits alpha5, alpha6, alphav, beta1, and beta5. Integrin function was investigated in iPSCs cultured without feeder layers on Matrigel or vitronectin, in comparison to human embryonic stem cells. beta1 integrins were required for adhesion and proliferation on Matrigel, as shown by immunological blockade experiments. On vitronectin, the integrin alphavbeta5 was required for initial attachment, but inhibition of both alphavbeta5 and beta1 was required to significantly decrease iPSC proliferation. Furthermore, iPSCs cultured on vitronectin for 9 passages retained normal karyotype, pluripotency marker expression, and capacity to differentiate in vitro. These studies suggest that vitronectin, or derivatives thereof, might substitute for Matrigel in a more defined system for iPSC culture.

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